U.S. FOOD & DRUG ADMINISTRATION

Bacteriological Analytical Manual

Chapter 5: *Salmonella*

October 2026 Edition

Table of Contents

Authors 1
Revision History 1
Introduction 4
A. Equipment and Materials 6
B. Media, Reagents, and Quality Control Cultures 7
C. Preparation of Foods for the Detection and Isolation of *Salmonella* 9
D. Screening of *Salmonella* 18
E. Isolation of *Salmonella* 19
F. Identification of *Salmonella* 21
References 29
Appendix A: Validated Food Matrices by FDA Loop-Mediated Isothermal Amplification (LAMP) and Real-Time Polymerase Chain Reaction (qPCR) Methods ... 32

Authors

Wallace H. Andrews (ret.), Hua Wang, Andrew Jacobson (ret.), Beilei Ge, Guodong Zhang, and Thomas Hammack (ret.)

Revision History

- October 2026: Corrected the date of the previous Revision History bullet to be January 2026 (there was no May 2025 edition) and expanded upon the revisions originally listed for that edition. Revised Appendix A title in the Table of Contents from "Validated food matrices" to "Validated Food Matrices by FDA Loop-Mediated Isothermal Amplification (LAMP) and Real-Time Polymerase Chain Reaction (qPCR) Methods" and added additional validated matrices for qPCR in Appendix A; Deleted mushroom from Section C7a and relocation to Section C7d; Revised Section C7d title from Fruits and vegetables to Fruits and vegetables, including mushroom, not otherwise specified in Section C; Added collagen hydrolysate to Section C.14 and created a new Section C.30 for whole onion; Correction of typo "25°C" to "2-5°C" in Section C21; Inserted correct hyperlinks for LAMP and qPCR screening methods in Section D and F; Updated the LAMP protocol in Section C.2 to include DNA template preparation from agar plates; Updated the qPCR protocol to include an optional wash step in Section E.1 and PrepMan™ Ultra reagent in Section E.2 for manual DNA extraction; Revised section F8h(2) from "discard cultures not confirmed as *Salmonella* by one of a, b, c, d, e, f or g methods" to "discard cultures not confirmed as *Salmonella* by a and b, or by one of c, d, e, f, or g methods"; Deleted “send to reference typing laboratory for definitive serotyping and identification” from Section F8(h)3; Removed shipping address from section F9 and F10; Deleted the second paragraph in Section F 10 and references 4 and 5.
- January 2026: Editorial revisions to sections A2, A9, and E1. Removed brand name for serological polyvalent flagellar (H) test in sections F4b and F4c. Renumbered original sections F9, F10, and F11 to F8, F9, and F10, respectively. Removed original section F9g; renumbered original sections F9h and F9i to F8g and F8h, respectively. Removed subsection designation "g" from original section F9i (1) (current F8h (1)). Revised original section F9i (2) (current F8h (2)) from "discard cultures not confirmed as *Salmonella* by a, and one of b, c, d or h methods" to "discard cultures not confirmed as *Salmonella* by one of a, b, c, d, e, f or g methods." Inserted reference 21 under section References. Section C.7, C.21, C.22, and C.26 updated to add same guidance for thawing method as C1; Section C.10 added flakes; Section C.24 revised preenrichment volume for swabs; Section D.1 updated LAMP protocol to include DNA extraction from presumptive *Salmonella* isolates (Section C) and baseline adjustment recommendations (Section F.3); Section D.2 updated qPCR protocol for the scope of manual DNA extraction methods application and revised the step 2 for quality check when preparing dehydrated master mix; and Appendix A added sealed concreated sampled with sponges as a new environmental matrix validated for LAMP.
- May 2024: Added a *Salmonella* screening section (Section D) to include generic LAMP and qPCR protocols; added a table (Appendix A) to list validated matrices by the two molecular methods; revamped Introduction to include a flow chart; updated Sections C.7 and C.24 on matrices and preenrichment broths including an alternative formula for modified buffered peptone water (M192c); deleted discontinued test kits in Section F.9. Original Section D and

Section E from all previous versions were shifted to Section E and Section F, respectively because of the addition of *Salmonella* screening Section D.

- September 2023: Section D.7 updated by requiring the use of both typical and atypical *Salmonella* culture controls.
- April 2023: Chapter 5 was converted to PDF and labeled as BAM Chapter 5: *Salmonella* (April 2023 Edition). Section C.28 updated to remove standing at room temperature and pH adjustment, which are steps not performed at the time of SLV and MLV.
- November 2022: Sections C.28 and E.9 updated to include screenshots for AB 7500 Fast setup and representative Genie instrument outputs, recommended GspSSD2.0 as the preferred LAMP master mix, and updated template preparation method to include a final centrifugation step. Updated section C.7.a to include oysters.
- March 2022: Section C.28 updated to include dairy feed as a validated animal food matrix, AB 7500 Fast as an alternative LAMP assay platform, two additional reagent choices for LAMP, and removed duplicate testing recommendation for LAMP screening of *Salmonella* in animal food protocol; Section E.9.d updated to include AB 7500 Fast as an alternative LAMP assay platform, two additional reagent choices for LAMP, and removed duplicate testing recommendation for LAMP confirmation of *Salmonella* isolates protocol; Section E.11 was updated by linking SeqSero to Protocols.IO.
- October 2021: Section C.2.c updated to correct a typo; Section E.11 serotyping submission information and shipping point of contact updated.
- September 2021: Section D.7 updated to include serological-formulas of ATCC control cultures 12325 and 29934; Section E.9.c updated to remove SmartCycler instructions from the Real-time PCR confirmation test.
- June 2021: Section A.27 updated to include ultraviolet light source; Section C.7 updated by changing nut/nut meat preparation procedure from blending to soaking; Section C.23 updated by removing erroneous requirement to adjust the pH of preenrichment before incubation; Section C.29 added method for spent sprout irrigation water from alfalfa, mung bean, and broccoli varieties; Section D.7 updated by adding Green Fluorescent Protein control strains.
- May 2021: Section C.28 updated to include an alternative positive control, a video demonstration, and software updates for LAMP screening for *Salmonella* in animal food protocol; Section E.9.d updated to include a video demonstration and software updates for LAMP confirmation of *Salmonella* isolates protocol; Section C.7.a updated to include mushrooms.
- February 2021: Removed text from Section E.9.b.
- December 2020: Minor changes in Sections D.10. TSI interpretation, Section E.9.b. use biochemical confirmation and typos in Section E.3.a, E.3.b, E.8 and Reference 2.
- July 2020: Section C.10. Added sample set up for non-powder forms of allspice, cinnamon, cloves, and oregano. Added reference 17.
- February 2020: Section C.28 added LAMP screening method for animal food.
- February 2020: Section E.9 added LAMP confirmation of *Salmonella* isolates protocol.
- December 2019: Section C.7 limited the foods covered by Section C.7 to those not listed elsewhere in Section C.

- November 2019: Section C.10.c added detailed procedure to prepare sample composites for oregano, cinnamon, allspice, and cloves.
- November 2019: Section E.9 updated to include additional options for identification of *Salmonella*.
- July 2018: Section C.7 revised to include vegetables not included in C.23 or C.27.
- March 2018: Added real-time quantitative PCR for confirmation of *Salmonella* isolates protocol and validated preenrichment changes for leafy produce, herbs and sprouts; Vegetables removed from revised Section C.7; Validated Preenrichment broth change in Section C.23.
- August 2016: Added the *Salmonella* Flipbook, a pictorial general guide to aid analysts in the detection and identification of *Salmonella* growing on the plating media and screening tubes used in the BAM Chapter 5 *Salmonella* method. See Section E.8. (Prepared By: Matthew J. Forstner, Laboratory Services, Minnesota Department of Agriculture). (PDF, 13Mb)
- December 2015: A section for the Statens Serum Institute Procedure was added to Section E: Identification of *Salmonella.*
- May 2014: The VITEK 2 method of presumptive generic identification of *Salmonella* was updated.
- February 2014: Section on detection and isolation of *Salmonella* from shell eggs was replaced, and validation data and additional references were added in an Appendix.
- August 2012: Made available in PDF format versions of Chapter 5: *Salmonella* and Appendix 1 (archived) from 2009 which were incorporated by reference in 21 CFR Parts 16 and 118: Federal Register Final Rule (July 9, 2009, 74 FR 33030): Prevention of *Salmonella* Enteritidis in Shell Eggs During Production, Storage, and Transportation.
- November 2011: Addition to Section C: Preparation of foods for isolation of *Salmonella*: Leafy green vegetables and herbs.
- February 2011: Removed link to Appendix 1: Rapid Methods for Detecting Foodborne Pathogens (now archived).
- December 2007: Mamey pulp method added, and Section D revised.
- June 2006: Eggs method revised for shell eggs and liquid whole eggs.
- April 2003: Frog legs method, lactic casein, rennet casein, sodium caseinate and rabbit carcass methods revised, top ears and other dog chew toys added. Removed Section A.25, Mechanical shaker.
- October 2001: Extension of the applicability of the orange juice method in Section C.19 to apple juice and apple cider.
- December 1999, March 2000, August 2000, and final revision on November 14, 2000. (see the Introduction Section for a summary of changes).

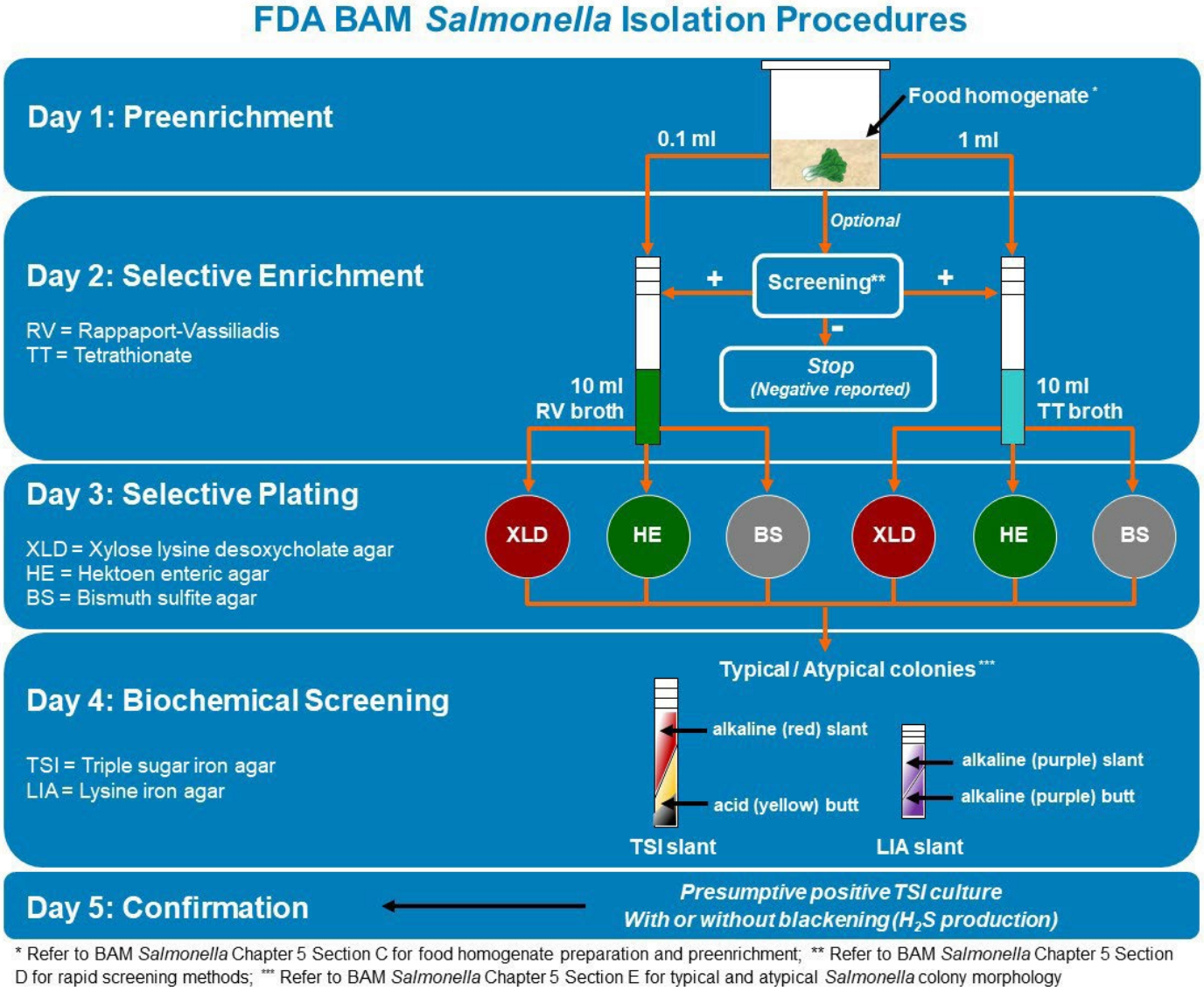
Introduction

Salmonella spp. are Gram-negative, non-spore-forming, usually motile, facultatively anaerobic, rod-shaped bacteria in the order *Enterobacterales,* family *Enterobacteriaceae*. The genus *Salmonella* consists of two species: *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is further divided into six subspecies: *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), and *S. enterica* subsp. *indica* (VI). Symbol "V" was retained for serovars of *S. bongori* [1]. Most of the *Salmonella* isolates that cause human infection belong to *S. enterica* subsp. *enterica* (I). *Salmonella* is a leading cause of foodborne illness in the United States. The foods involved in *Salmonella* outbreaks include meat and poultry products, fruits and vegetables, eggs, milk, nuts, peanut butter, and spices, etc. *Salmonella* is also recognized as a major microbial hazard in animal food, which includes pet food, animal feed, and raw materials and ingredients.

The *Bacteriological Analytical Manual* (BAM) *Salmonella* culture method is used to recover *Salmonella* from a broad range of human food and animal food matrices that FDA regulates. The method has been continually updated with method development and improvement research following the Guidelines for the Validation of Microbiological Methods for the FDA Foods Program and approved by the FDA's Microbiological Methods Validation Subcommittee (MMVS). The updates are listed in the Revision History in chronological order.

For general sampling and sample homogenate preparation, refer to BAM Chapter 1: Food Sampling and Preparation of Sample Homogenate, and for *Salmonella* detection in poultry house environmental samples, refer to "Environmental Sampling and Detection of *Salmonella* in Poultry Houses". For the enumeration of *Salmonella*, refer to "Most Probable Number Determination from Serial Dilutions" in BAM Appendix 2.

The BAM *Salmonella* culture method takes 4~5 days for a presumptive result. As shown in the flow chart, the FDA BAM *Salmonella* isolation procedures consist of sample preparation and preenrichment (day 1), selective enrichment (day 2), selective plating (day 3), biochemical screening (day 4), and confirmation (day 5). Two FDA-developed molecular methods, a loop-mediated isothermal amplification (LAMP) method and a real-time PCR (qPCR) method may be used to rapidly screen samples for the presence of *Salmonella* in 24-h preenrichment cultures on day 2. With either screening method, only samples determined to be positive will be confirmed by culture procedures unless molecular detection is inhibited. If the molecular methods give indeterminant results, then the culture method is performed until completion. The protocols and validated food matrices for these two screening methods can be found in Section D and Appendix A.



* Refer to BAM *Salmonella* Chapter 5 Section C for food homogenate preparation and preenrichment; ** Refer to BAM *Salmonella* Chapter 5 Section D for rapid screening methods; *** Refer to BAM *Salmonella* Chapter 5 Section E for typical and atypical *Salmonella* colony morphology

BAM *Salmonella* analysis requires the use of both typical and atypical *Salmonella* strains for positive controls. A non-*Salmonella* strain negative control and an uninoculated media control are also needed for sample analysis. Refer to Section B.56 for Suggested Quality Control Cultures.

A. Equipment and Materials

1. Blender and sterile blender jars
2. Sterile, homogenizer blender filter bags (710 ml) or equivalent, sterile 500 ml wide-mouth, screw-cap jars, sterile 500 ml Erlenmeyer flasks, sterile 250 ml beakers, sterile glass or paper funnels of appropriate size, and, optionally, containers of appropriate capacity to accommodate large and composited samples
3. Sterile, bent glass or plastic spreader rods
4. Balance, 2000 g capacity, sensitivity of 0.1 g
5. Balance, 120 g capacity, sensitivity of 5 mg
6. Incubator, 35 ± 2°C
7. Refrigerated incubator or laboratory refrigerator, 4 ± 2°C
8. Water bath, 49 ± 1°C
9. Water bath, circulating, thermostatically-controlled, 42 ± 0.2°C and 43 ± 0.2°C
10. Sterile spoons or other appropriate instruments for transferring food samples
11. Sterile culture dishes, 15 × 100 mm, glass or plastic
12. Sterile pipets, 1 ml, with 0.01 ml graduations; 5 and 10 ml, with 0.1 ml graduations
13. Sterile inoculating needle and inoculating loop (10 µl and 1 µl)
14. Sterile test or culture tubes, 16 × 150 mm and 20 × 150 mm; serological tubes, 10 × 75 mm or 13 × 100 mm
15. Test or culture tube racks
16. Vortex mixer
17. Sterile shears, large scissors, scalpel, and forceps
18. Fisher or Bunsen burner
19. pH test paper (pH range 6-8) with maximum graduations of 0.4 pH units per color change or pH meter
20. Plastic bags, 28 × 37 cm, sterile, with resealable tape (Items 21-22 are needed in the analysis of frog legs and rabbit carcasses.)
21. Autoclavable plastic beakers for holding plastic bags during shaking and incubation
22. Sponges, non-bactericidal, or equivalent
23. Swabs, non-bactericidal, cotton-tipped
24. Ultraviolet (UV) light source with an emission wavelength between 360 to 400 nm when Green Fluorescent Protein (GFP)-UV control strains are used.

B. Media, Reagents, and Quality Control Cultures

1. Lactose broth (M74)
2. Nonfat dry milk (reconstituted) (M111)
3. Selenite cystine (SC) broth (M134)
4. Tetrathionate (TT) broth (M145)
5. Rappaport-Vassiliadis (RV) medium (M132) NOTE: RV medium must be made rom its individual ingredients. Commercial formulations are not acceptable.
6. Xylose lysine desoxycholate (XLD) agar (M179)
7. Hektoen enteric (HE) agar (M61)
8. Bismuth sulfite (BS) agar (M19)
9. Triple sugar iron agar (TSI) (M149)
10. Tryptone (tryptophane) broth (M164)
11. Trypticase (tryptic) soy broth (M154)
12. Trypticase soy-tryptose broth (M160)
13. MR-VP broth (M104)
14. Simmons citrate agar (M138)
15. Urea broth (M171)
16. Urea broth (rapid) (M172)
17. Malonate broth (M92)
18. Lysine iron agar (LIA) (Edwards and Fife) (M89
19. Lysine decarboxylase broth (M87)
20. Motility test medium (semisolid) (M103)
21. Potassium cyanide (KCN) broth (M126)
22. Phenol red carbohydrate broth (M121)
23. Purple carbohydrate broth (M130)
24. MacConkey agar (M91)
25. Nutrient broth (M114)
26. Brain heart infusion (BHI) broth (M24)
27. Papain solution, 5% (M56a)
28. Cellulase solution, 1% (M187)
29. Tryptose blood agar base (M166)

30. Universal preenrichment broth (M188)
31. Universal preenrichment broth (without ferric ammonium citrate) (M188a)
32. Buffered peptone water (M192)
33. Dey-Engley broth (M193)
34. Potassium sulfite powder, anhydrous
35. Chlorine solution, 200 ppm, containing 0.1% sodium dodecyl sulfate (R12a)
36. Ethanol, 70% (R23)
37. Kovacs' reagent (R38)
38. Voges-Proskauer (VP) test reagents (R89)
39. Creatine phosphate crystals
40. Potassium hydroxide solution, 40% (R65)
41. 1 N Sodium hydroxide solution (R73)
42. 1 N Hydrochloric acid (R36)
43. Brilliant green dye solution, 1% (R8)
44. Bromcresol purple dye solution, 0.2% (R9)
45. Methyl red indicator (R44)
46. Sterile distilled water
47. Tergitol anionic 7 (R78)
48. Triton X-100 (R86)
49. Physiological saline solution, 0.85% (sterile) (R63)
50. Formalinized physiological saline solution (R27)
51. *Salmonella* polyvalent somatic (O) antiserum
52. *Salmonella* polyvalent flagellar (H) antiserum
53. *Salmonella* somatic group (O) antisera: A, B, C_1, C_2, C_3, D_1, D_2, E_1, E_2, E_3, E_4, F, G, H, I, Vi, and other groups, as appropriate
54. *Salmonella* Spicer-Edwards flagellar (H) antisera
55. Modified buffered peptone water, two formulas (M192b, M192c)
56. **Suggested Quality Control Cultures**

    Analysts must use both typical and atypical *Salmonella* controls for sample analysis, since it may be more difficult to confirm the presence of an atypical strain in a sample without an atypical control strain for guidance.

    Green fluorescent protein (GFP) control strains which fluoresce under UVA light, more specifically at wavelengths between 360 to 400 nm, have been developed by FDA and have been licensed by FDA to Microbiologics for distribution. (https://www.microbiologics.com; 200 Cooper Avenue North St. Cloud, MN 56303, 1800-599-2847). The following cultures may be purchased from Microbiologics:

- *Salmonella enterica* subsp. *enterica* serovar Gaminara Sal57 / FDA SAL5695 (typical, lactose negative, H_2S positive), Catalog No. 01278UV.
- *Salmonella enterica* subsp. *enterica* serovar Senftenberg Sal59 / FDA Sal5697 (atypical, lactose negative, H_2S negative), Catalog No. 01226UV.
- *Salmonella enterica* subsp. *enterica* serovar Mbandaka Sal58 / FDA SAL5696 (atypical, sucrose positive, H_2S negative), Catalog No. 01230UV.

Non-GFP fluorescent *Salmonella* control strains may also be used for sample analysis. In addition to the positive control cultures (typical *Salmonella*; lactose negative, H_2S positive), 3 additional *Salmonella* cultures are recommended to assist in the selection of atypical *Salmonella* colony morphology on selective agars. These cultures are:

- Lactose positive, H_2S-positive *S. diarizonae* (47:i:z53:z57, ATCC 12325)
- Lactose-negative, H_2S-negative *S.* Abortusequi (4,12:-:e,n,x, ATCC 9842)
- Lactose-positive, H_2S-negative *S. diarizonae* (60:r:e,n,x,z15, ATCC 29934)

These cultures may be obtained from the American Type Culture Collection (www.atcc.org),10801 University Boulevard, Manassas, VA 20110-2209.

C. Preparation of Foods for the Detection and Isolation of *Salmonella*

The following methods for sample homogenate preparation and preenrichment are based on the analysis of a 25 g analytical unit at a 1:9 sample/broth ratio. Depending on the extent of compositing, add enough broth to maintain this 1:9 ratio unless otherwise indicated. For samples not analyzed on an exact weight basis, e.g., frog legs, refer to the specific method for instructions.

1. **Dried egg yolk, dried egg whites, dried whole eggs, liquid milk (skim milk, 2% fat milk, whole, and buttermilk), and prepared powdered mixes (cake, cookie, doughnut, biscuit, and bread), infant formula, and oral or tube feedings containing egg.** Preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw suitable portion as rapidly as possible to minimize increase in number of competing organisms or to reduce potential of injuring *Salmonella* organisms. Thaw below 45°C for 15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5°C. Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. For nonpowdered samples, add 225 ml sterile lactose broth. If product is powdered, add about 15 ml sterile lactose broth and stir with sterile glass rod, spoon, or tongue depressor to smooth suspension. Add 3 additional portions of lactose broth, 10, 10, and 190 ml, for total of 225 ml. Stir thoroughly until sample is suspended without lumps. Cap jar securely and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2 with sterile 1 N NaOH or 1 N HCl. Cap jar securely and mix well before determining final pH. Loosen jar cap about 1/4 turn and incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

2. **Eggs**

   a. **Shell eggs** [13,14]. Eggs with chipped, cracked, or broken shells are not included in the sample. Remove any adherent material from the shell eggs surface. Disinfect egg surface with a solution consisting of 3 parts of 70% alcohol (ethyl or isopropyl) to 1 part iodine/potassium iodide solution. Prepare 70% alcohol solution either by diluting 700 ml 100% alcohol with sterile distilled water for a final volume of 1,000 ml or by diluting 700 ml 95% alcohol with sterile distilled water for a final volume of 950 ml. Prepare iodine/potassium iodide solution by dissolving 100 g potassium iodide in 200-300 ml sterile distilled water. Add 50 g iodine and heat gently with constant mixing until the iodine is dissolved. Dilute the iodine/potassium iodide solution to 1,000 ml with sterile distilled water. Store iodine/potassium iodide solution in an amber glass-stoppered bottle in the dark if not used immediately. Prepare the disinfection solution by adding 250 ml iodine/potassium iodide solution to 750 ml 70% alcohol solution and mix well. Submerge eggs in disinfection solution for 10 seconds (make sure not less than 10 seconds). Remove eggs from the solution and allow to air dry. Each sample shall consist of twenty (20) eggs, for a total of fifty (50) samples per poultry house. Eggs are cracked aseptically into a 4-L sterile beaker or other suitable container by gloved hands, with a change of gloves between samples. Mix samples thoroughly with a sterile tool by gloved hands until yolks are completely mixed with the albumen, with a change of gloves between samples. Preenrich the 20-egg sample by adding 2 L sterile trypticase soy broth (TSB; room temperature) and mix well with a sterile tool. Cover securely and incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

   b. **Liquid whole eggs (homogenized).** Combine fifteen (15) 25 ml test portions into a 375 ml composite contained in a 6-liter Erlenmeyer flask. Composites are held at room temperature (20-24°C) for 96 ± 2 h. After 96 ± 2 h, add 3,375 ml sterile trypticase soy broth (TSB) supplemented with ferrous sulfate, as described above, and mix well by swirling. Let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

   c. **Hard-boiled eggs (chicken, duck, and others).** Aseptically separate the shells from the egg contents. Pulverize the egg contents (egg yolk solids and egg white solids) aseptically and weigh 25 g into a sterile 500 ml Erlenmeyer flask or other appropriate container. Add 225 ml trypticase soy broth (TSB) (without ferrous sulfate) and mix well by swirling. Continue as in E.1-11.

3. **Nonfat dry milk**

   a. **Instant**. Aseptically weigh 25 g sample into sterile beaker (250 ml) or other appropriate container. Using sterile glass or paper funnel (made with tape to withstand autoclaving), pour 25 g analytical unit gently and slowly over surface of 225 ml brilliant green water contained in sterile 500 ml Erlenmeyer flask or other appropriate container. Alternatively, 25 g analytical units may be composited and poured over the surface of proportionately larger volumes of brilliant green water. Prepare brilliant green water by adding 2 ml 1% brilliant green dye solution per 1000 ml sterile distilled water. Let container stand undisturbed for 60 ± 5 min.

Incubate loosely capped container, without mixing or pH adjustment, for 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

b. **Non-Instant**. Examine as described for instant nonfat dry milk, except that the 25 g analytical units may not be composited.

4. **Dry whole milk**. Examine as described for instant nonfat dry milk, except that the 25 g analytical units may not be composited.

5. **Casein**

   a. **Lactic casein.** Aseptically weigh 25 g sample into sterile beaker (250 ml) or other appropriate container. Using sterile glass or paper funnel (made with tape to withstand autoclaving), pour 25 g analytical unit gently and slowly over the surface of 225 ml universal preenrichment broth (UPB) contained in sterile 500 ml Erlenmeyer flask or other appropriate container. Analytical units (25 g) may be composited. Let container stand undisturbed 60 ± 5 min. Incubate loosely capped container, without mixing or pH adjustment, for 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

   b. **Rennet casein.** Aseptically weigh 25 g sample into sterile beaker (250 ml) or other appropriate container. Using sterile glass or paper funnel (made with tape to withstand autoclaving), pour 25 g analytical unit gently and slowly over the surface of 225 ml lactose broth contained in sterile 500 ml Erlenmeyer flask or other appropriate container. Analytical units (25 g) may be composited. Let container stand undisturbed 60 ± 5 min. Incubate loosely capped container, without mixing or pH adjustment, for 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

   c. **Sodium caseinate**. Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile lactose broth and mix well. Analytical units may be composited. Let stand 60 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Loosen jar about 1/4 turn and incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

6. **Soy flour**. Examine as described for rennet casein, except 25 g analytical units (25 g) may not be composited.

7. **Fresh, frozen, or dried products.** Preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw suitable portion as rapidly as possible to minimize increase in number of competing organisms or to reduce potential of injuring *Salmonella* organisms. Thaw below 45°C for <15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5°C.

   a. **Egg-containing products (noodles, egg rolls, macaroni, spaghetti), crustaceans (shrimp, crab, crayfish, langostinos, lobster), fish and oysters.** Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile universal preenrichment broth (UPB) and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

b. **Nuts and nut meats.** Aseptically weigh 25 g sample into a sterile wide mouth Erlenmeyer flask or other appropriate container. Add 225 ml universal preenrichment broth (UPB) and mix well by swirling. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

c. **Nut butter.** Aseptically weigh 25 g sample into a sterile wide mouth Erlenmeyer flask or other appropriate container. Add 225 ml universal preenrichment broth (UPB) and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Mix well and loosen jar cap about ¼ turn. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

d. **Fruits and vegetables, including mushrooms, not otherwise specified in Section C.** Aseptically weigh 25 g sample into a sterile wide mouth Erlenmeyer flask or other appropriate container. Add 225 ml universal preenrichment broth (UPB) and mix well by swirling. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

e. **Cheese, dough, prepared salads (ham, egg, chicken, tuna, turkey).** Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile lactose broth and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

8. **Dried yeast (active and inactive yeast)**. Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile trypticase soy broth (TSB). Mix well to form smooth suspension. Let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2, mixing well before determining final pH. Loosen jar cap 1/4 turn and incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

9. **Frosting and topping mixes**. Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml nutrient broth and mix well. Cap jar securely and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Loosen jar cap about 1/4 turn and incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

10. **Spices**

a. **Black pepper, white pepper, celery seed or flakes, chili powder or flakes, cumin, paprika, parsley flakes, rosemary, sesame seed, thyme, and vegetable flakes**. Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile trypticase soy broth (TSB) and mix well. Cap jar securely and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Loosen jar cap about l/4 turn and incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

b. **Onion flakes, onion powder, garlic flakes**. Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Preenrich sample in trypticase soy broth (TSB) with added K_2SO_3 (5 g K_2SO_3 per 1000 ml TSB, resulting in final 0.5% K_2SO_3 concentration). Add K_2SO_3 to broth before autoclaving 225 ml volumes in 500 ml Erlenmeyer flasks at 121°C for 15 min. After autoclaving, aseptically determine and, if necessary, adjust final volume to 225 ml. Add 225 ml sterile TSB with added K_2SO_3 to sample and mix well. Continue as in C.10.a.

c. **Allspice, cinnamon, cloves, and oregano**. There are no known methods for neutralizing the toxicity of these 4 spices (powder or non-powder) at this time. Dilute spices beyond their toxic levels to examine them for the presence of *Salmonella*. Examine allspice, cinnamon, and oregano at 1:100 sample/broth ratio, and cloves at 1:1000 sample/broth ratio.

   In more detail, for oregano, cinnamon, and allspice, mix each sample thoroughly; take 25.0 g from each of 15 subsamples for a total of 375.0 g. Thoroughly mix this composite. Then from the 375.0 g composite, measure 37.5 g, and add to 3712.5 ml of sterile pre-enrichment broth (TSB). Shake and mix well for preenrichment. For food categories requiring 30 subsamples, create 2 sets of composites, each consisting of 15 subsamples; for those requiring 60 subsamples, create 4 sets of composites, each consisting of 15 subsamples.

   Then follow the procedures for 15 subsamples described above. For cloves, create a 375.0 g composite from 15 subsamples according to the procedures described above. Then from the 375.0 g composite, measure 3.75 g, and add to 3746.25 ml of sterile pre- enrichment broth (TSB). Shake and mix well for pre-enrichment. A minimum of 37.5 g sample size is required, 10 tests should be conducted from the same 375.0 g composite. For food categories requiring 30 subsamples, create 2 sets of composites, each consisting of 15 subsamples; for those requiring 60 subsamples, create 4 sets of composites, each consisting of 15 subsamples. Then follow the procedures for 15 subsamples described above.

   After the pre-enrichment procedures described above, examine these spices as described in C.10.a, above, maintaining recommended sample/broth ratios.

   Non-powder forms of allspice, cinnamon, cloves, and oregano (whole, chunks, pieces, leaves). Aseptically weigh 25 g sample into a sterile Whirl-Pak® filter bag or equivalent. Add 225 ml TSB and vigorously shake for 60 sec manually.

   Transfer the rinsate immediately into a fresh sterile bag. **It is crucial to add TSB into the sample immediately before shaking and to transfer the rinsate out immediately after shaking.** If the samples are very absorptive of broth (such as oregano leaves), 475ml of TSB should be added to 25 g of samples. Continue to pre-enrich the rinsate as in C.10.a.

11. **Candy and candy coating (including chocolate)**. Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile, reconstituted nonfat dry milk and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Add 0.45 ml 1% aqueous brilliant green dye solution and mix

well. Loosen jar caps 1/4 turn and incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

12. **Coconut**. Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile lactose broth, shake well, and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Add up to 2.25 ml steamed (15 min) Tergitol anionic 7 and mix well. Alternatively, use steamed (15 min) Triton X-100. Limit use of these surfactants to minimum quantity needed to initiate foaming. For Triton X-100 this quantity may be as little as 2 or 3 drops. Loosen jar cap about l/4 turn and incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

13. **Food dyes and food coloring substances**. For dyes with pH 6.0 or above (10% aqueous suspension), use method described for dried whole eggs in C.I, above. For laked dyes or dyes with pH below 6.0, aseptically weigh 25 g sample into sterile, wide-mouth, screwcap jar (500 ml) or other appropriate container. Add 225 ml tetrathionate broth without brilliant green dye. Mix well and let stand 60 ± 5 min at room temperature with jar securely capped. Using pH meter, adjust pH to 6.8 ± 0.2. Add 2.25 ml 0.1% brilliant green dye solution and mix thoroughly by swirling. Loosen jar cap about 1/4 turn and incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

14. **Gelatin, collagen hydrolysate**. Aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile lactose broth and 5 ml 5% aqueous papain solution and mix well. Cap jar securely and incubate at 35 ± 2°C for 60 ± 5 min. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Loosen jar cap about 1/4 turn and incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11 below.

15. **Meats, meat substitutes, meat by-products, animal substances, glandular products, and meals (fish, meat, bone)**. Aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile lactose broth and blend 2 min. Aseptically transfer homogenized mixture to sterile wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. If mixture is powder or is ground or comminuted, blending may be omitted. For samples that do not require blending, add lactose broth and mix thoroughly; let stand for 60 ± 5 min at room temperature with jar securely capped.

    Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Add up to 2.25 ml steamed (15 min) Tergitol anionic 7 and mix well. Alternatively, use steamed (15 min) Triton X-100. Limit use of these surfactants to minimum quantity needed to initiate foaming. Actual quantity will depend on composition of test material. Surfactants will not be needed in analysis of powdered glandular products. Loosen jar caps 1/4 turn and incubate sample mixtures 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

16. **Frog legs.** (This method is used for all domestic and imported frog legs.) Place 15 pairs of frog legs into sterile plastic bag and cover with sterile lactose broth at a 1:9 sample-to-broth (g/ml) ratio (**see** A, 23-24, above). If single legs are estimated to average 25 g or more, examine only one leg of each of 15 pairs. Place bag in large plastic beaker or other suitable container. Mix well and let stand 60 ± 5 min at room temperature. Mix well

by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Place plastic bag containing the frog legs and lactose broth into plastic beaker or other suitable container. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

17. **Rabbit carcasses.** (This method is used for all domestic and imported rabbit carcasses.) Place rabbit carcass into sterile plastic bag. Place bag in beaker or other suitable container. Add sterile lactose broth at a 1:9 sample-to-broth (g/ml) ratio to cover carcass (see A, 23-24, above). Mix well by swirling and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

18. **Guar gum**. Aseptically weigh 25 g sample into sterile beaker (250 ml) or other appropriate container. Prepare a 1.0% cellulase solution (add 1 g cellulase to 99 ml sterile distilled water). Dispense into 150 ml bottles. (Cellulase solution may be stored at 2-5°C for up to 2 weeks). Add 225 ml sterile lactose broth and 2.25 ml sterile 1% cellulase solution to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. While vigorously stirring the cellulase/lactose broth with magnetic stirrer, pour 25 g analytical unit quickly through sterile glass funnel into the cellulase/lactose broth. Cap jar securely and let stand 60 ± 5 min at room temperature. Incubate loosely capped container without pH adjustment, for 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

19. **Orange juice (pasteurized and unpasteurized), apple cider (pasteurized and unpasteurized), and apple juice (pasteurized)**. Aseptically add 25 ml sample to 225 ml universal preenrichment broth (UPB) in a sterile, wide mouth, screw-capped jar (500 ml) or other appropriate container. Swirl the flask contents thoroughly. Cap jar securely and let stand 60 ± 5 min at room temperature. Do not adjust pH. Incubate loosely capped container for 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below (treat as a low microbial load food).

20. **Pig ears and other types of dog chew pieces.** Place 1 piece (or 2-3 pieces if smaller sizes) from each sample unit into sterile plastic bag. Place bag into large beaker or other suitable container. Add sterile lactose broth at a 1:9 sample-to-broth (g/ml) ratio to cover pieces (see A, 23-24, above). Mix well by swirling and let stand 60 ± 5 min at room temperature. Mix well by swirling and determine pH with test paper. Adjust pH, if necessary, to 6.8 ± 0.2. Add either steamed (15 min) Tergitol anionic 7 or steamed (15 min) Triton X-100 up to a 1% concentration. For example, if 225 ml lactose broth is added, the maximum volume of added surfactant is 2.25 ml. Limit use of these surfactants to minimum quantity to initiate foaming. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

21. **Cantaloupes**. Preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw suitable portion as rapidly as possible to minimize increase in number of competing organisms or to reduce potential of injuring *Salmonella* organisms. Thaw below 45°C for <15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5°C.

    For comminuted or cut fruit, aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile universal preenrichment broth (UPB) and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Do not adjust pH. Mix well and loosen jar cap about 1/4 turn. Incubate

24 ± 2 h at 35 ± 2°C. Continue as in E.1-11.

For whole cantaloupes, do not rinse even if there is visible dirt. Examine the cantaloupes "as is".

Place the cantaloupe into a sterile plastic bag. Add enough UPB broth to allow the cantaloupe to float. The volume of UPB broth may be 1.5 times the weight of the cantaloupes. For example, cantaloupes weighing 1500 g will probably need a volume of approximately 2250 ml UPB broth to float. Add more broth, if necessary. Place the plastic bag, with cantaloupes and UPB broth, into a 5 liter beaker, or other appropriate container, for support during incubation. Allow the open-end flap of the plastic bag to "fold over" so as to form a secure, but not air-tight, closure during incubation.

Let stand for 60 ± 5 min at room temperature. Do not adjust pH. Incubate slightly opened bag, containing cantaloupe, for 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

22. **Mangoes**. Preferably, do not thaw frozen samples before analysis. If frozen sample must be tempered to obtain analytical portion, thaw suitable portion as rapidly as possible to minimize increase in number of competing organisms or to reduce potential of injuring *Salmonella* organisms. Thaw below 45°C for <15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5°C.

    For comminuted or cut fruit, aseptically weigh 25 g sample into sterile blending container. Add 225 ml sterile buffered peptone water (BPW) and blend 2 min.

    Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Mix well by swirling and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

    For whole mangoes, do not rinse even if there is visible dirt. Examine the mangoes "as is".

    Place the mango into a sterile plastic bag. Add enough BPW to allow the mango to float. The volume of BPW may be 1.0 times the weight of the mangoes. For example, mangoes weighing 500 g will probably need a volume of approximately 500 ml BPW broth to float. Add more broth, if necessary. Place the plastic bag, with mangoes and BPW broth, into a 5-liter beaker, or other appropriate container, for support during incubation.

    Let stand for 60 ± 5 min at room temperature. Incubate slightly opened bag for 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

23. **Tomatoes**. For comminuted or cut fruit, aseptically weigh 25 g sample into sterile blending container. Add 225 ml universal preenrichment broth (UPB) and blend 2 min. Aseptically transfer homogenized mixture to sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container and let stand 60 ± 5 min at room temperature with jar securely capped. Do not adjust pH. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

    For whole tomatoes, do not rinse even if there is visible dirt. Examine the tomatoes "as is".

    Place the tomato into a sterile plastic bag or other suitable container (sterile foil covered beaker can be used). Add enough UPB broth to allow the tomato to float. The volume of UPB broth may be 1.0 times the weight of the tomato. For example, tomatoes weighing

300 g will probably need a volume of approximately 300 ml UPB broth to float. Add more, if necessary. Place the plastic bag (if used), with tomato and UPB broth, into a sterile beaker (beaker size is dependent on the size of the tomato), or other appropriate container, for support during incubation. Allow the open-end flap of the plastic bag to "fold over" so as to form a secure, but not air-tight, closure during incubation.

Let stand for 60 ± 5 min at room temperature. Do not adjust pH. Incubate slightly opened bag for 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

24. **Environmental testing**. Sample environmental surfaces with sterile swabs or sponges following manufacturer's swab/sponge sampling instruction. Place the swab/sponge in a sterile Whirl-Pak® bag, or equivalent, that contains approximately 10 ml Dey-Engley (D/E) neutralizing broth to cover the swab/sponge.

    Transport swabs/sponges in an insulated transport container with frozen gel packs to keep the samples cold, but not frozen. If samples cannot be processed immediately, refrigerate at 4 ± 2°C. Start sample analysis within 48 ± 2 h of collection.

    For swab/sponge submerged in approximately 10 ml D/E neutralizing broth, add the entire contents to 90 ml modified Buffered Peptone Water (mBPW, M192c) in a sterile, wide-mouth, screw-capped jar (500 ml) or another appropriate sterile container. Sponge in D/E neutralizing broth can be also added to 225 ml mBPW (M192c).Ensure contents are mixed thoroughly and incubate 24 ± 2 h at 35 ± 2°C. Continue examination as in E.1-11, below.

25. **Alfalfa seeds and mung beans**. Aseptically weigh 25g alfalfa seeds or mung beans into a sterile 500 ml Erlenmeyer flask. Aseptically add 225 ml lactose broth to the test portion and swirl the Erlenmeyer flask. Cover the mouth of the Erlenmeyer flask with sterile aluminum foil and allow contents to stand at room temperature for 60 ± 5 min.

    Adjust the pH of the culture to 6.8 ± 0.2, if necessary. Incubate for 24 ± 2h at 35 ± 2°C. Continue as in E.1-11, below (treat as high microbial load food).

26. **Mamey pulp**. If frozen, sample must be tempered to obtain analytical portion, thaw suitable portion as rapidly as possible to minimize increase in number of competing organisms or to reduce potential of injuring *Salmonella* organisms. Thaw below 45 ± 0.2°C for <15 min with continuous agitation in thermostatically controlled water bath or thaw within 18 h at 2-5°C.

    For mamey pulp, suspected to be contaminated with *S*. Typhi, aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile universal preenrichment broth (UPB) without ferric ammonium citrate, mix by swirling, and let stand 60 ± 5 min at room temperature with jar securely capped. Do not adjust pH. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below. Treat as a low microbial load food.

    For mamey pulp, NOT suspected to be contaminated with *S*. Typhi, aseptically weigh 25 g sample into sterile, wide-mouth, screw-cap jar (500 ml) or other appropriate container. Add 225 ml sterile universal preenrichment broth (UPB), mix by swirling, and let stand 60 ± 5 min at room temperature with jar securely capped. Do not adjust pH. Mix well and loosen jar cap about 1/4 turn. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

27. **Fresh leafy green vegetables, herbs and sprouts (baby spinach, cabbage, iceberg lettuce, Romaine lettuce, Spring mix, basil, cilantro, dill, curly parsley, culantro,**

Italian parsley, watercress, alfalfa, mung bean, clover, radish and broccoli sprouts). Aseptically weigh 25 g into a sterile wide mouth Erlenmeyer flask or other appropriate container. Add 225 ml universal preenrichment broth (UPB) (for cabbage, adding 225 ml modified buffered peptone water (mBPW, M192b) and completely soak contents without any homogenization. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

28. **Animal food.** Aseptically weigh 25 g sample into a sterile sample filter bag. Place the bag into a large container or rack for support during incubation. Add 225 ml sterile buffered peptone water (BPW). Mix well by swirling and briefly hand massage for 2 min. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below.

29. **Spent sprout irrigation water from alfalfa, mung bean, and broccoli varieties.** Aseptically add 375 ml sample to 1,125 ml universal preenrichment broth (UPB) in a sterile Whirl-Pak filter bag, or equivalent. Place bag into large container or rack for support during incubation. Mix well by swirling. Incubate 24 ± 2 h at 35 ± 2°C. Continue examination as in E.1-11, below (treat as a high microbial load food).

30. **Whole onions.** Do not rinse even if there is visible dirt. Examine the onions "as is". Aseptically cut onion in half and place contents into a sterile plastic bag. Add enough either universal preenrichment broth (UPB) or trypticase soy broth (TSB) with added K_2SO_3 (5 g K_2SO_3 per 1000 mL TSB, resulting in final 0.5% K_2SO_3 concentration; Add K_2SO_3 to broth before autoclave broth) This volume of UPB or TSB should be approximately 1.5 times the weight of the onion. Add more UPB or TSB, if necessary to keep the onion fully immersed. Place the plastic bag, with onion and broth, into a non-sterile 5-liter beaker, or other appropriate container, for support during incubation. Allow the open-end flap of the plastic bag to “fold over” so as to form a secure, but not air-tight, closure during incubation. Incubate 24 ± 2 h at 35 ± 2°C. Continue as in E.1-11, below (treat as a high microbial load food).

D. Screening of *Salmonella*

Following preenrichment, either FDA LAMP or qPCR method, may be used to screen *Salmonella* in 24-h preenrichment cultures before isolation of *Salmonella* in Section E.

1. **LAMP screening method:** *Salmonella* LAMP protocol (PDF, 2.42 MB; Contact: Beilei.Ge@fda.hhs.gov).
2. **qPCR screening method:** *Salmonella* qPCR protocol (PDF, 650 KB; Contact: Hua.Wang@fda.hhs.gov).

Refer to Appendix A for the list of validated food matrices and environmental samples by the two FDA rapid screening methods. When using these two methods for food matrices not listed in the table, verification or validation will be needed following the Guidelines for the Validation of Microbiological Methods for the FDA Foods Program.

Samples that have negative rapid screening results will be reported as negative, and no further culture analysis will be required. Samples that have positive rapid screening results must be confirmed culturally following the isolation and identification procedures described in Section E and Section F, below.

For samples with rapid screening results that are suspicious of inhibition effects, a repeat rapid screen with diluted DNA template will be needed. For a matrix spike used in a sample analysis, if the matrix spike has a rapid screening negative result, the sample must be continued with culture analysis following the isolation and identification procedures described in Section E and Section F, below.

E. Isolation of *Salmonella*

The following *Salmonella* isolation procedures consist of selective enrichment, selective plating, and biochemical screening.

1. After preenrichment, gently shake incubated sample:

   Guar gum and foods suspected to be contaminated with *S.* Typhi. Transfer 1 ml mixture to 10 ml selenite cystine (SC) broth and another 1 ml mixture to 10 ml Tetrathionate (TT) broth. Vortex.

   All other foods. Transfer **0.1** ml mixture to 10 ml Rappaport-Vassiliadis (RV) medium and another 1 ml mixture to 10 ml TT broth. Vortex.

2. Incubate selective enrichment media as follows:

   Foods with a high microbial load. Incubate RV medium 24 ± 2 h at 42 ± 0.2°C (circulating, thermostatically-controlled, water bath). Incubate TT broth 24 ± 2 h at 43 ± 0.2°C (circulating, thermostatically controlled, water bath).

   Foods with a low microbial load (except guar gum and foods suspected to be contaminated with *S.* Typhi). Incubate RV medium 24 ± 2 h at 42 ± 0.2°C (circulating, thermostatically controlled, water bath). Incubate TT broth 24 ± 2 h at 35 ± 2°C.

   Guar gum and foods suspected to be contaminated with *S.* Typhi. Incubate SC and TT broths 24 ± 2 h at 35 ± 2°C.

3. Mix (vortex, if tube) and streak 3 mm loopful (10 µl) incubated TT broth on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. **Prepare BS plates the day before streaking and store in dark at room temperature until streaked.**

4. Repeat with 3 mm loopful (10 µl) of RV medium (for samples of high and low microbial load foods) and of SC broth (for guar gum) on XLD, HE, and BS agars.

5. Refer to 994.04 in *Official Methods of Analysis* [2] for option of refrigerating incubated sample preenrichments and incubated sample selective enrichments (SC and TT broths only) of low moisture foods (LMF) for up to 72 h. LMF have a water activity of less than or equal to 0.85 such as nuts, dried fruits, cereal products and chocolate etc. This option allows sample analyses to be initiated as late as Thursday while still avoiding weekend work.

6. Incubate plates 24 ± 2 h at 35 ± 2°C.

7. Examine plates for the presence of colonies that may be *Salmonella*.

Salmonella Flipbook, a pictorial general guide may be used to aid analysts in the detection and identification of *Salmonella* growing on the plating media and screening slants (prepared by Matthew J. Forstner, Laboratory Services, Minnesota Department of Agriculture).

Typical *Salmonella* colony morphology

Pick 2 or more colonies of *Salmonella* from each selective agar plate after 24 ± 2 h incubation. Typical *Salmonella* colonies are as follows:

a. **Hektoen enteric (HE) agar**. Blue-green to blue colonies with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies.

b. **Xylose lysine desoxycholate (XLD) agar**. Pink colonies with or without black centers. Many cultures of *Salmonella* may produce colonies with large, glossy black centers or may appear as almost completely black colonies.

c. **Bismuth sulfite (BS) agar**. Brown, gray, or black colonies; sometimes they have a metallic sheen. Surrounding medium is usually brown at first, but may turn black in time with increased incubation, producing the so-called halo effect.

If typical colonies are present on the BS agar after 24 ± 2 h incubation, then pick 2 or more colonies. Irrespective of whether or not BS agar plates are picked at 24 ± 2 h, reincubate BS agar plates an additional 24 ± 2 h. After 48 ± 2 h incubation, pick 2 or more typical colonies, if present, from the BS agar plates, only if colonies picked from the BS agar plates incubated for 24 ± 2 h give atypical reactions in triple sugar iron agar

(TSI) and lysine iron agar (LIA) that result in culture being discarded as not being *Salmonella*. **See** Sections E.9 and E.10, below, for details in interpreting TSI and LIA reactions.

Atypical *Salmonella* colony morphology

In the absence of typical or suspicious *Salmonella* colonies, search for atypical *Salmonella* colonies as follows:

a. **HE and XLD agars**. Atypical *Salmonella* cultures produce yellow colonies with or without black centers on HE and XLD agars. In the absence of typical *Salmonella* colonies on HE or XLD agars after 24 ± 2 h incubation, then pick 2 or more atypical *Salmonella* colonies.

b. **BS agar.** Atypically some strains produce green colonies with little or no darkening of the surrounding medium. If typical or suspicious colonies are not present on BS agar after 24 ± 2 h, then do not pick any colonies but reincubate an additional 24 ± 2 h. If typical or suspicious colonies are not present after 48 ± 2 h incubation, then pick 2 or more atypical colonies.

8. Lightly touch the very center of the colony to be picked with sterile inoculating needle and inoculate TSI slant by streaking slant and stabbing butt. Without flaming, inoculate LIA slant by stabbing butt twice and then streaking slant. Since lysine decarboxylation reaction is strictly anaerobic, the LIA slants must have deep butt (4 cm). Store picked selective agar plates at 5-8°C.

9. Incubate TSI and LIA slants at 35 ± 2°C for 24 ± 2 h. Cap tubes loosely to maintain aerobic conditions while incubating slants to prevent excessive H_2S production. *Salmonella* in culture typically produces alkaline (red) slant and acid (yellow) butt, with or without production of H_2S (blackening of agar) in TSI. In LIA, *Salmonella* typically produces alkaline (purple) reaction in butt of tube. Consider only distinct yellow in butt of tube as acidic (negative) reaction. Do not eliminate cultures that produce discoloration in butt of tube solely on this basis. Most *Salmonella* cultures produce H_2S in LIA. Some non- *Salmonella* cultures produce a brick-red reaction in LIA slants.

10. Cultures that give an alkaline butt in LIA should be retained as potential *Salmonella* isolates and submitted for biochemical and serological tests if the TSI slant is either alkaline over acid or acid over acid. Cultures that give an acid butt in LIA and an alkaline slant and acid butt in TSI should also be considered potential *Salmonella* isolates and should be submitted for biochemical and serological tests. Cultures that give an alkaline butt in LIA, but where there is no change in the TSI for both the slant and the butt should be discarded. Cultures that give an acid butt in LIA and an acid slant and acid butt in TSI may be discarded as not being *Salmonella*. Test retained, presumed-positive TSI cultures as directed in E.11, below, to determine if they are *Salmonella* species, including *S. arizonae*. If TSI cultures fail to give typical reactions for *Salmonella* (alkaline slant and acid butt) pick additional suspicious colonies from selective medium plate not giving presumed-positive culture and inoculate TSI and LIA slants as described in E.8, above.

11. Apply biochemical and serological identification tests to:

    a. Three presumptive TSI cultures recovered from set of plates streaked from RV medium (or SC broth for guar gum), if present, and 3 presumptive TSI agar cultures recovered from plates streaked from TT broth, if present.

    b. If 3 presumptive-positive TSI cultures are not isolated from one set of agar plates, test other presumptive-positive TSI agar cultures, if isolated, by biochemical and serological tests. Examine a minimum of 6 TSI cultures for each 25 g analytical unit or each 375 g composite.

F. Identification of *Salmonella*

The following identification procedures of the presumptive *Salmonella* isolates from TSI slant in E.11 consist of purification, serological tests, biochemical tests, confirmation using official methods, whole genome sequencing (WGS) and SeqSero analysis for serotype identification.

1. **Mixed cultures**. Streak TSI agar cultures that appear to be mixed on XLD agar , HE agar, or MacConkey agar. Incubate plates 24 ± 2 h at 35 ± 2°C. Examine plates for presence of colonies suspected to be *Salmonella*.

    a. **Hektoen enteric (HE) agar**. **See** E.7.a, above.

    b. **Xylose lysine desoxycholate (XLD) agar**. **See** E.7.b, above. Transfer at least 2 colonies suspected to be *Salmonella* to TSI and LIA slants as described in E.7, above, and continue as in E.9, above.

    c. **MacConkey agar**. Typical colonies appear transparent and colorless, sometimes with dark center. Colonies of *Salmonella* will clear areas of precipitated bile caused by other organisms sometimes present.

2. **Pure cultures**

   a. **Urease test (conventional)**. With sterile needle, inoculate growth from each presumed-positive TSI slant culture into tubes of urea broth. Since occasional, uninoculated tubes of urea broth turn purple-red (positive test) on standing, include uninoculated tube of this broth as control. Incubate 24 ± 2 h at 35 ± 2°C.

   b. **Optional urease test (rapid)**. Transfer two 3-mm loopfuls of growth from each presumed-positive TSI slant culture into tubes of rapid urea broth. Incubate 2 h in 37 ± 0.5°C water bath. Discard all cultures giving positive test. Retain for further study all cultures that give negative test (no change in color of medium).

3. **Serological somatic (O) tests for *Salmonella*.** (Pre-test all antisera to *Salmonella* with known cultures)

   a. **Polyvalent somatic (O) test**. Using wax pencil, mark off 2 sections about 1 × 2 cm each on inside of glass or plastic petri dish (15 × 100 mm). Commercially available sectioned slides may be used. Emulsify 3 mm loopful of culture from 24-48 h TSI slant or, preferably, tryptose blood agar base (without blood) with 2 ml 0.85% saline. Add 1 drop of culture suspension to upper portion of each rectangular crayon-marked section. Add 1 drop of saline solution to lower part of one section only. Add 1 drop of *Salmonella* polyvalent somatic (O) antiserum to other section only. With clean sterile transfer loop or needle, mix culture suspension with saline solution for one section and repeat for other section containing antiserum. Tilt mixtures in back-and-forth motion for 1 min and observe against dark background in good illumination. Consider any degree of agglutination a positive reaction. Classify polyvalent somatic (O) test results as follows:

      Positive — agglutination in test mixture; no agglutination in saline control.

      Negative — no agglutination in test mixture; no agglutination in saline control.

      Nonspecific — agglutination in test and in control mixtures. Perform further biochemical and serological tests as described in *Edwards and Ewing's Identification of Enterobacteriaceae* [3].

   b. **Somatic (O) group tests**. Test as in E.6a, above, using individual group somatic

      (O) antisera including Vi, if available, in place of *Salmonella* polyvalent somatic

      (O) antiserum. For special treatment of cultures giving positive Vi agglutination reaction, refer to sec. 967.28B in *Official Methods of Analysis* [2]. Record cultures that give positive agglutination with individual somatic (O) antiserum as positive for that group. Record cultures that do not react with individual somatic (O) antiserum as negative for that group.

4. **Serological polyvalent flagellar (H) test**

   a. Perform the polyvalent flagellar (H) test at this point, or later, as described in F.5 below. Inoculate growth from each urease-negative TSI agar slant into either 1) BHI broth and incubate 4-6 h at 35 ± 2°C until visible growth occurs (to test on same day); or 2) trypticase soy-tryptose broth and incubate 24 ± 2 h at 35 ± 2°C (to test on following day). Add 2.5 ml formalinized physiological saline solution to 5 ml of either broth culture.

b. Tube agglutination test. Select 2 formalinized broth cultures and test with *Salmonella* polyvalent flagellar (H) antisera per antisera manufacturer's instructions. Place 0.5 ml of appropriately diluted *Salmonella* polyvalent flagellar (H) antiserum in 10 × 75 mm or 13 × 100 mm serological test tube. Add 0.5 ml formalinized antigen to be tested. Prepare saline control by mixing 0.5 ml physiological saline solution with 0.5 ml formalinized antigen. Incubate mixtures in 48-50°C water bath. Observe at 15 min intervals and read final results in 1 h.

Positive — agglutination in test mixture and no agglutination in control.

Negative — no agglutination in test mixture and no agglutination in control.

Nonspecific — agglutination in both test mixture and control. Test the cultures giving such results with Spicer-Edwards antisera.

c. Slide agglutination test. Perform the polyvalent flagellar (H) test per antisera manufacturer's instructions. *Salmonella* is grown over night on a non-selective agar medium. Swarm agar is the best suited medium for growing cultures for H typing, but H antigens can be serotyped from a non-selective agar medium if the H antigens are well expressed. Add a small drop of antiserum (approx. 20 µL) on a glass slide or plastic petri dish (15 × 100 mm). Transfer culture using an inoculating loop from several colonies to the drop of antiserum and mix well. The amount of culture should be sufficient to give a distinct milky turbidity. Tilt the slide or petri dish for 5-10 seconds. A positive reaction is seen as visible agglutination, whereas a negative reaction is seen as homogeneous milky turbidity. Late or weak agglutination should be considered negative. Physiological saline (0.85%, pH 7.4) is used as a negative control and must be negative.

5. Spicer-Edwards serological test. Use this test as an alternative to the polyvalent flagellar (H) test. It may also be used with cultures giving nonspecific agglutination in polyvalent flagellar (H) test. Perform Spicer-Edwards flagellar (H) antisera test as described in F.4.b, above. Perform additional biochemical tests (F.6.a-c, below) on cultures giving positive flagellar test results. If both formalinized broth cultures are negative, perform serological tests on 4 additional broth cultures (F.4.a, above). If possible, obtain 2 positive cultures for additional biochemical testing F.6.a-c, below). If all urease-negative TSI cultures from sample give negative serological flagellar (H) test results, perform additional biochemical tests F.6.a-c, below).

6. Testing of urease-negative cultures

a. **Lysine decarboxylase broth.** If LIA test was satisfactory, it need not be repeated. Use lysine decarboxylase broth for final determination of lysine decarboxylase if culture gives doubtful LIA reaction. Inoculate broth with small amount of growth from TSI slant suspicious for *Salmonella*. Replace cap tightly and incubate 48 ± 2 h at 35 ± 2°C but examine at 24 ± 2 h intervals. *Salmonella* species cause alkaline reaction indicated by purple color throughout medium. Negative test is indicated by yellow color throughout medium. If medium appears discolored (neither purple nor yellow) add a few drops of 0.2% bromcresol purple dye and re-read tube reactions.

b. **Phenol red dulcitol broth or purple broth base with 0.5% dulcitol**. Inoculate broth with small amount of growth from TSI culture. Replace cap loosely and incubate 48 ± 2 h at 35 ± 2°C but examine after 24 ± 2 h. Most *Salmonella*

species give positive test, indicated by gas formation in inner fermentation vial and acid pH (yellow) of medium. Production of acid should be interpreted as a positive reaction. Negative test is indicated by no gas formation in inner fermentation vial and red (with phenol red as indicator) or purple (with bromcresol purple as indicator) color throughout medium.

c. **Tryptone (or tryptophane) broth.** Inoculate broth with small growth from TSI agar culture. Incubate 24 ± 2 h at 35 ± 2°C and proceed as follows:

1. **Potassium cyanide (KCN) broth.** Transfer 3 mm loopful of 24-h tryptophane broth culture to KCN broth. Heat rim of tube so that good seal is formed when tube is stoppered with wax-coated cork. Incubate 48 ± 2 h at 35 ± 2°C but examine after 24 ± 2 h. Interpret growth (indicated by turbidity) as positive. Most *Salmonella* species do not grow in this medium, as indicated by lack of turbidity.

2. **Malonate broth.** Transfer 3 mm loopful of 24-h tryptone broth culture to malonate broth. Since occasional uninoculated tubes of malonate broth turn blue (positive test) on standing, include uninoculated tube of this broth as control. Incubate 48 ± 2 h at 35 ± 2°C but examine after 24 ± 2 h. Most *Salmonella* species cultures give negative test (green or unchanged color) in this broth.

3. **Indole test.** Transfer 5 ml of 24-h tryptophane broth culture to empty test tube. Add 0.2-0.3 ml Kovacs' reagent. Most *Salmonella* cultures give negative test (lack of deep red color at surface of broth). Record intermediate shades of orange and pink as ±.

4. **Serological flagellar (H) tests for *Salmonella*.** If either polyvalent flagellar (H) test (F.4, above) or the Spicer-Edwards flagellar (H) test tube test (F.5, above) has not already been performed, either test may be performed here.

5. Discard as not *Salmonella* any culture that shows either positive indole test and negative serological flagellar (H) test, or positive KCN test and negative lysine decarboxylase test.

7. **Additional biochemical tests**. Classify as *Salmonella* those cultures which exhibit typical *Salmonella* reactions for tests 1-11, shown in Table 1. If one TSI culture from 25 g analytical unit is classified as *Salmonella*, further testing of other TSI cultures from the same 25 g analytical unit is unnecessary. Cultures that contain demonstrable *Salmonella* antigens as shown by positive *Salmonella* flagellar (H) test but do not have biochemical characteristics of *Salmonella* should be purified (F.1, above) and retested, beginning with F.2, above.

Perform the following additional tests on cultures that do not give typical *Salmonella* reactions for tests 1-11 in **Table 1** and that consequently do not classify as *Salmonella*.

a. **Phenol red lactose broth or purple lactose broth.**

1. Inoculate broth with small amount of growth from unclassified 24-48 h TSI slant. Incubate 48 ± 2 h at 35 ± 2°C but examine after 24 ± 2 h.

   Positive — acid production (yellow) and gas production in inner fermentation vial. Consider production of acid only as positive reaction.

Most cultures of *Salmonella* give negative test result, indicated by no gas formation in inner fermentation vial and red (with phenol red as indicator) or purple (with bromcresol purple as indicator) throughout medium.

2. Discard as not *Salmonella*, cultures that give positive lactose tests, except cultures that give acid slants in TSI and positive reactions in LIA, or cultures that give positive malonate broth reactions. Perform further tests on these cultures to determine if they are *S. arizonae.*

b. **Phenol red sucrose broth or purple sucrose broth.** Follow procedure described in F.7a.1, above. Discard as not *Salmonella*, cultures that give positive sucrose tests, except those that give acid slants in TSI and positive reactions in LIA.

c. **MR-VP broth.** Inoculate medium with small amount of growth from each unclassified TSI slant suspected to contain *Salmonella*. Incubate 48 ± 2 h at 35 ± 2°C.

1. Perform Voges-Proskauer (VP) test at room temperature as follows: Transfer 1 ml 48h culture to test tube and incubate remainder of MR-VP broth an additional 48 ± 2 h at 35 ± 2°C. Add 0.6 ml α-naphthol and shake well. Add 0.2 ml 40% KOH solution and shake. To intensify and speed reaction, add a few crystals of creatine. Read results after 4 h; development of pink-to-ruby red color throughout medium is positive test. Most cultures of *Salmonella* are VP-negative, indicated by absence of development of pink-to-red color throughout broth.

2. Perform methyl red test as follows: To 5 ml of 96-h MR-VP broth, add 5-6 drops of methyl red indicator. Read results immediately. Most *Salmonella* cultures give a positive test, indicated by diffuse red color in medium. A distinct yellow color is a negative test. Discard, as not *Salmonella*, cultures that give positive KCN and VP tests and negative methyl red test.

d. **Simmons citrate agar**. Inoculate this agar, using needle containing growth from unclassified TSI agar slant. Inoculate by streaking slant and stabbing butt. Incubate 96 ± 2 h at 35 ± 2°C. Read results as follows:

Positive — presence of growth, usually accompanied by color change from green to blue. Most cultures of *Salmonella* are citrate-positive.

Negative — no growth or very little growth and no color change.

e. **Classification of cultures**. Classify, as *Salmonella*, cultures that have reaction patterns of Table I. Discard, as not *Salmonella*, cultures that give results listed in any subdivision of Table 2. Perform additional tests described in *Edwards and Ewing's Identification of Enterobacteriaceae* (3) to classify any culture that is not clearly identified as *Salmonella* by classification scheme in Table 1 or not eliminated as not being *Salmonella* by test reactions in Table 2. If neither of 2 TSI cultures carried through biochemical tests confirms the isolate as *Salmonella*, perform biochemical tests, beginning with F.6, on remaining urease-negative TSI cultures from same 25 g analytical unit.

Table 1. Biochemical and serological reactions of *Salmonella*

#	Test or substrate	Positive Result	Negative Result	*Salmonella* species reaction[a]
1.	Glucose (TSI)	yellow butt	red butt	+
2.	Lysine decarboxylase (LIA)	purple butt	yellow butt	+
3.	H_2S (TSI and LIA)	blackening	no blackening	+
4.	Urease	purple-red color	no color change	−
5.	Lysine decarboxylase broth	purple color	yellow color	+
6.	Phenol red dulcitol broth	yellow color and/or gas	no gas; no color change	+[b]
7.	KCN broth	growth	no growth	−
8.	Malonate broth	blue color	no color change	−[c]
9.	Indole test	red color at surface	yellow color at surface	−
10.	Polyvalent flagellar test	agglutination	no agglutination	+
11.	Polyvalent somatic test	agglutination	no agglutination	+
12.	Phenol red lactose broth	yellow color and/or gas	no gas; no color change	−[c]
13.	Phenol red sucrose broth	yellow color and/or gas	no gas; no color change	−
14.	Voges-Proskauer test	pink-to-red color	no color change	−
15.	Methyl red test	diffuse red color	diffuse yellow color	+
16.	Simmons citrate	growth; blue color	no growth; no color change	v

[a] +: 90% or more positive in 1 or 2 days; −: 90% or more negative in 1 or 2 days; v: variable.
[b] Majority of *S. arizonae* cultures are negative.
[c] Majority of *S. arizonae* cultures are positive.

Table 2. Criteria for discarding non-*Salmonella* cultures

#	Test or substrate	Results
1.	Urease	positive (purple-red color)
2.	Indole test and Polyvalent flagellar (H) test; or Indole test and Spicer-Edwards flagellar test	positive (red color at surface) negative (no agglutination) positive (red color at surface) negative (no agglutination)
3.	Lysine decarboxylase and KCN broth	negative (yellow color) positive (growth)
4.	Phenol red lactose broth	positive (yellow color and/or gas)[a], [b]
5.	Phenol red sucrose broth	positive (yellow color and/or gas)[b]
6.	KCN broth, Voges-Proskauer test, and Methyl red test	positive (growth) positive (pink-to-red color) negative (diffuse yellow color)

[a] Test malonate broth positive cultures further to determine if they are *S. arizonae*.
[b] Do not discard positive broth cultures if corresponding LIA cultures give typical *Salmonella* reactions; test further to determine if they are *Salmonella* species.

8. **Official confirmation methods for presumptive *Salmonella* isolates.**

   a. **Serological confirmation:** perform the *Salmonella* serological somatic (O) test (F.3.a, above) and the *Salmonella* serological flagellar (H) test (F.4, above) or the Spicer-Edwards flagellar (H) test (F.5, above). Serological confirmation must always be paired with biochemical confirmation.

   b. **Biochemical confirmation:** Instead of the conventional biochemical tube system above, API 20E, The Enterotube™ II, or Vitek 2 GN (AOAC OMA 2011.17) may be used. **Commercial biochemical kits should not be used as a substitute for serological tests** [2].

   c. **Real-time PCR (qPCR) confirmation test:** *Salmonella* qPCR protocol (PDF, 650 KB; Contact: Hua.Wang@fda.hhs.gov).

   d. **Loop-mediated isothermal amplification (LAMP) confirmation test:**

      Salmonella LAMP protocol (PDF, 2.42 MB; Contact: Beilei.Ge@fda.hhs.gov).

   e. **ANSR® *Salmonella* confirmation test (AOAC OMA method 2013.14):** using isothermal nucleic acid amplification assay based on the nicking enzyme amplification reaction (NEAR) technology for the identification and confirmation of *Salmonella*.

   f. **Bruker MALDI Biotyper Method (AOAC OMA method 2017.09):** using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) for the identification and confirmation of bacteria.

g. **Other instrumental confirmation methods validated per the Guidelines for the Validation of Microbiological Methods for the FDA Foods Program, AOAC's Appendix J, or ISO 16140-6:2019 are acceptable if approved for use by FDA's Microbiology Methods Validation Subcommittee.**

h. **Classification of cultures:**

1. Report as *Salmonella* those cultures classified as *Salmonella* by a and b, or by one of c, d, e, f, or g methods.
2. Discard cultures not confirmed as *Salmonella* by a and b, or by one of c, d, e, f, or g methods.
3. For cultures having conflicted confirmation by above methods, classify according to additional tests specified in F. 2-7, above, or additional tests as specified by Ewing [3].

9. **Treatment of cultures giving negative flagellar (H) test**.

If biochemical reactions of certain flagellar (H)-negative culture strongly suggest that it is *Salmonella*, the negative flagellar agglutination may be the result of nonmotile organisms or insufficient development of flagellar antigen. Proceed as follows: Inoculate motility test medium in petri dish, using small amount of growth from TSI slant. Inoculate by stabbing medium once about 10 mm from edge of plate to depth of 2-3 mm. Do not stab to bottom of plate or inoculate any other portion. Incubate 24 ± 2 h at 35 ± 2°C. If organisms have migrated 40 mm or more, retest as follows: Transfer 3 mm loopful of growth that migrated farthest to Trypticase Soy-Tryptose Broth. Repeat either polyvalent flagellar (H) (F.4, above) or Spicer-Edwards (F.5, above) serological tests. If cultures are not motile after the first 24 ± 2 h, incubate an additional 24 ± 2 h at 35 ± 2°C; if still not motile, incubate up to 5 days at 25°C. Classify culture as nonmotile if above tests are still negative.

10. **Submission of cultures for WGS and serotyping**. Submit 1 isolate of each somatic group recovered from each analytical unit, unless otherwise instructed. Isolate(s) should be submitted to your local serotyping group for WGS and SeqSero analysis.

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Appendix A: Validated food matrices by FDA loop-mediated isothermal amplification (LAMP) and real-time polymerase chain reaction (qPCR) methods

Sample preparation reference in Section C	Food matrices validated by LAMP	Food matrices validated by qPCR
C.1	All-purpose flour, cake mixes, cookies, egg powder, infant cereal, raw milk, whey-based infant formula	Powdered infant formula (casein-based and soy-based), All-purpose flour, cake mixes, cookies, egg powder, infant cereal, raw milk, whey-based infant formula
C.2	Liquid egg, shell eggs	Egg noodle, shell eggs, Liquid egg
C.3	Nonfat dehydrated milk	Nonfat dehydrated milk
C.7	Frozen shrimp, frozen tilapia, ice cream, peanut butter, smoked fish, soft cheese	Almond, almond butter, blackberry, blueberry, breakfast cereal, chia seeds, peanut butter, pine nuts, raspberry, strawberry, walnuts, frozen shrimp, frozen tilapia, ice cream, smoked fish, soft cheese
C.10	Ground black pepper	Crushed red pepper, ground basil, ground black pepper, ground cumin, ground white pepper, paprika, red chili powder
C.11	Cocoa powder	Cocoa powder
C14	NA	Collagen Hydrolysate
C.20	Pig ear	NA
C.21	Cantaloupe	Cantaloupe
C.24	Stainless-steel surface sampled with swabs, sealed concrete surface sampled with sponges	Cast iron, ceramic tile, plastic, rubber, and stainless-steel surfaces sampled with swabs and sponges
C.27	Alfalfa sprouts, romaine lettuce	Alfalfa sprouts, baby spinach, basil, cabbage, cilantro, dill, lettuce, oregano, parsley, spring mix, watercress
C.28	Animal feed (cattle feed, dairy feed, horse feed, poultry feed, swine feed), pet food (dry cat food, dry dog food,raw beef pet food, raw chicken pet food)	Dry chick feed, animal feed ingredients (dry premium alfalfa pellets, dry wheat bran, dry whole oats)
C.29	Recycled washing water from different food manufacturers	Sprout spent irrigation water, recycled washing water from different food manufacturers
C.30	NA	Whole onions

NA: Not Available